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# CAROTENE CONTENT OF THE CORN PLANT [1]

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## INTRODUCTION

Corn silage ranks with hay as a principal winter roughage for dairy cattle in Wisconsin and adjacent States. It is becoming recognized that content of carotene, the parent substance of vitamin A, is one of the important factors affecting the quality of these winter feeds and of the milk produced thereon. Beginning in 1938, a study was undertaken at this station of the factors that influence the carotene content of hybrid corn (*Zea mays* L.). An initial objective was a determination of the extent to which different inbred lines and the hybrids derived from them varied inherently in capacity to form this substance. On the answer to this question rested the possibility of improving the carotene content of corn by selective breeding.

To this end a large number of genetically pure inbred, single hybrid, and double hybrid strains of corn were raised and their carotene content determined at various stages of growth. During the summer of 1940, for example, approximately 27 inbred, 18 single hybrid, and 30 double hybrid strains were examined. Continuation of the study through four growing seasons has provided data from which the effect of environmental variations, as contrasted with genetic make-up, may be estimated. The effect on carotene content of such factors as the sun-red gene, the sugary gene (sweet corn), the number of plants per hill, and the prevention of pollination were also studied, as was the distribution of the pigment in various parts of the corn plant.

In view of the unique collection of experimental material made available during this work, it was considered advisable to extend the analytical determination to xanthophyll and chlorophyll. The present report, however, is concerned entirely with carotene values, and represents a greatly condensed summary of the complete results which are too extensive to be published in full.[3]

## REVIEW OF LITERATURE

Only a few investigations have been made to determine the variation in carotene content among different strains of a particular plant species. Emsweller, Burrell, and Borthwick (*3*) [4] examined 18 lines

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[2] The authors are indebted to B. Schweigert, S. Hanrahan, E. Gangstad, and C. Owens for technical and field assistance.

[3] The complete data are available in the doctoral dissertation of John W. Porter, University of Wisconsin, 1942.

[4] Italic numbers in parentheses refer to Literature Cited, p. 187.

of carrots and found from 32 to 63 mg. of carotene per 100 gm. of dry weight. Later Johnson and Miller (*5*) analyzed the grain of 19 inbred lines of corn and found that significant variations occurred. They reported a close relationship between the number of genes for yellow endosperm color and the concentration of total carotenoids and β-carotene.

Virtanen (*14*) reported that the total quantity of carotene in various plants increased rapidly up to the time of flowering and then diminished as the plant matured. Murneek (*9*) analyzed plants of three species which were grown under long and short day conditions. He found that the short day (reproducing) plants had a higher carotene concentration than the long day (vegetative) plants. Further, analyses of soybean leaves over a portion of the growing season showed a maximum carotene concentration at the time of flowering. Esselen and coworkers (*4*) found that the vitamin A potency of corn increased as the plant reached full growth, after which there was a considerable decrease. Moon (*6*) reported a marked loss, after flowering, in the carotene content of grasses. Snyder and Moore (*12*) analyzed corn leaves from tasseling time to maturity and found the maximum concentration 2 weeks after tasseling. Nagasima (*9*) reported that the concentration of the carotenoids in seed-bearing plants showed two maxima, the first during the period of most active growth and the second at the time of sexual reproduction.

The effect of season on carotene concentration apparently has not been studied with the exception of determinations of the amount present in pasture grasses during spring, summer, and fall. However, a number of observations on the effect of fertilization, moisture, light intensity, and temperature have been reported. Virtanen (*14*) found that all factors which have an unfavorable influence on the growth of plants, such as soil acidity, excessive concentrations of phosphate, potassium, sodium chloride, etc., lower the content of carotene in the plant. Also he found that carotene concentration is increased by the application of fertilizers. Pfützer and Pfaff-Limburgerhof (*10*) found that nitrogen fertilization always increased the carotene content of the plants they studied. Moon (*7*) reported that nitrogen and potassium each increased the carotene content of pasture grass growing on poor soil. Nitrogen fertilization produced the greatest increase.

Moon (*6*) reported that drought conditions reduced the carotene concentration of grasses. Atkeson, Peterson, and Aldous (*1*) found many pasture grasses increased in carotene content following fall rains.

Barnes (*2*) found that maximum color developed in Chantenay carrots when they were grown at 15° to 21° C. Smith (*11*) found the carotenoid content of greenhouse-grown tomato fruits to be less than that of the same variety grown out of doors. He reported also that some of the shorter wave lengths of light increase the carotenoid content of fruits.

## EXPERIMENTAL METHODS

### PREPARATION OF SAMPLE

In preparation for the carotene analyses eight plants of a strain were harvested at the desired stage of development. This number of plants was taken for each sample throughout the study. In 1939 the entire leaf (sheath and blade) was separated from the remainder of

the plant, but in the following 2 years only the leaf blade, minus the midrib, was removed from the rest of the plant. This change was made so that small strain differences could be determined more accurately. Leaf blades are relatively homogeneous tissue which contain 65 to 80 percent of the carotene and only about 10 percent of the total dry weight of the entire plant.

Each year the two fractions were separately chopped into ⅛- to ¼-inch pieces with a Gehl ensilage cutter. Chopping rather than grinding was used because, as the values in table 1 show, there is a consider-

TABLE 1.—*Carotene concentration of chopped and ground corn leaf samples*

| Description of sample | Method of preparation | |
|---|---|---|
| | Chopped (⅛ to ¼-inch lengths) | Chopped and then ground (electric meat grinder) |
| | *Micrograms per gram* | *Micrograms per gram* |
| Greenhouse-grown sweet corn | 394 | 208 |
| | 286 | 264 |
| | 227 | 199 |
| Field-grown sweet corn | 611 | 274 |
| | 575 | 264 |
| Field-grown single hybrid | 463 | 323 |
| | | 269 |

able loss of carotene from green corn plants when the latter method is employed.

### EXTRACTION OF CAROTENE

After cutting, each fraction was weighed, mixed thoroughly, and duplicate 50-gm. leaf samples and a single 100-gm. sample of the remainder material were taken for carotene analysis. Duplicate 100-gm. samples of each fraction were also taken for moisture determinations. The samples for carotene analyses were immediately introduced into extraction flasks and covered with hot alcohol in order to avoid the rather slight destruction of carotene which was found to occur when cold alcohol was used or when the samples were allowed to stand before the addition of alcohol.

Removal of carotene from the leaf samples was accomplished in three successive extractions by refluxing with 300 to 400-cc. portions of 95-percent ethyl alcohol and decanting the extracts. Samples of the remainder of the plant were extracted three times with 200 to 300-cc. portions of alcohol. Negligible quantities of carotene were removed by further extraction.

### STORAGE OF EXTRACTS

The volume of the combined extracts of each sample was measured and a 25-cc. aliquot was taken for carotene and xanthophyll determinations. A 10-cc. aliquot was also taken for chlorophyll determination. The 25-cc. aliquot was introduced into a small bottle (capacity 200 to 250 cc.) and 5 cc. of 10-percent alcohol KOH was added. The solution was saturated with nitrogen, the bottle stoppered, and the sample stored in the dark at −5° C. until analyzed, from 1 to 6 months later. The data in table 2, which refer to longer storage at a higher

temperature, support the belief that there was no loss in carotene content during the storage period.

TABLE 2.—*Stability of carotene during storage of alcoholic extracts of corn* [1]

| Strain or variety | Analyses performed immediately after extraction | Analyses performed 9 months after extraction |
|---|---|---|
| | *Micrograms per gram* | *Micrograms per gram* |
| Golden Glow | 70 | 72 |
| 645 | 79 | 72 |
| 570 | 61 | 55 |
| 525 | 63 | 70 |
| Funk hybrid 235 | 103 | 108 |

[1] The alcoholic extracts were stored under $N_2$ at room temperature in the dark, and contained KOH.

## SEPARATION AND DETERMINATION OF CAROTENE

Carotene and xanthophyll were removed from the alcohol solution by diluting with 30 cc. of water and shaking with 15-cc. portions of Skellysolve B until the latter removed no more color. The combined Skellysolve B fractions were washed once with 30 cc. of water and then extracted with 15-cc. portions of 85-percent ethyl alcohol until the latter came off colorless. The Skellysolve B solution containing the carotene fraction from the leaf material was made to 100 cc. In the case of the stalk material, which was much lower in carotene,

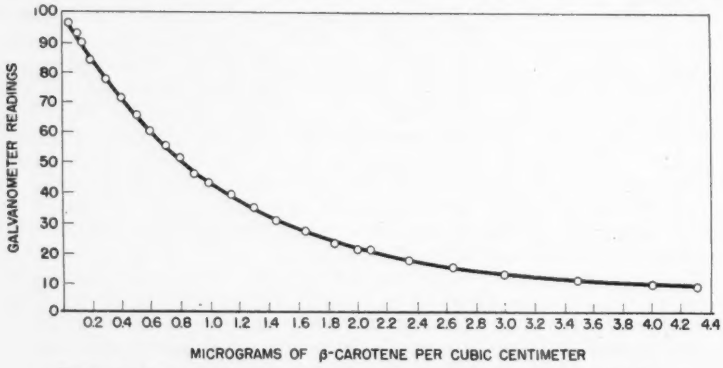


FIGURE 1.—Curve showing absorption of light by β-Carotene in Skellysolve B solution.

the Skellysolve B solution was diluted to 50 cc. The dilution factor for the calculation was therefore 2 instead of 4 as given in the formula below. The pigment content of the Skellysolve B solutions was determined with an Evelyn photoelectric colorimeter equipped with a 440 μ filter. The results were expressed in terms of micrograms of carotene per cubic centimeter by interpolation of the Evelyn readings on a previously constructed standard curve (fig. 1). A Skellysolve B solution of pure β-carotene [5] was used to obtain this curve.

[5] Obtained from General Biochemicals, Chagrin Falls, Ohio.

In order to determine whether any carotene was being destroyed during the analysis, Skellysolve B solutions of carotene were allowed to stand exposed to light and air at room temperature for a period of 29 hours. From the data in table 3 it can be seen that there was no loss in carotene during that time.

TABLE 3.—*Stability of carotene in Skellysolve solutions exposed to light and air at room temperature*

| Strain or variety | Evelyn galvanometer readings | |
|---|---|---|
| | Original value | Value after 29 hours |
| Golden Glow | 82.8 | 82.8 |
| $K_{13} \times M_{13}$ | 72.3 | 72.3 |
| $8\text{-}29 \times A_{48}$ | 78.3 | 77.5 |
| 14 | 77.5 | 78.0 |
| $A_{48}$ | 78.3 | 78.3 |
| 645 | 84.5 | 84.5 |

The amount of carotene per gram of dry matter was calculated by the following formula:

$$\frac{\text{Micrograms of carotene per cc.} \times 4 \times \text{total volume of extract}}{\text{grams of dry weight of the sample}}$$

All the results in the following sections are expressed on the dry basis and unless otherwise stated refer only to leaf material from which the leaf sheath and midrib were removed.

## RESULTS

### DISTRIBUTION OF CAROTENE IN THE CORN PLANT

To determine the variation in the carotene concentration of different parts of the plant separate analyses were made on the leaves, stalks, husks, and ears of one strain. The values in table 4 show the

TABLE 4.—*Carotene concentration in different parts of the corn plant*

| Part of plant [1] | Carotene content at— | | Fraction of total dry weight at— | |
|---|---|---|---|---|
| | Anthesis | Early dent stage | Anthesis | Early dent stage |
| | *Micrograms per gram* | *Micrograms per gram* | *Percent* | *Percent* |
| Leaves | 836 | 359 | 9 | 7 |
| Stalks | 53 | 8 | 91 | |
| Husks | | 10 | | 93 |
| Ears | | 7 | | |

[1] The analyses were made on strain $R_4$.

concentration of carotene and the percentage of the total dry weight present in these parts of individual plants at two stages of growth. The carotene concentration is so high in the leaves that they contain about two-thirds to three-fourths of the total, although comprising less than one-tenth of the dry weight.

Sprague and Curtis (*13*) made analyses of different leaves on the same corn plant and reported that carotene decreased rather regularly from the uppermost to the lowest leaves. The writers repeated this experiment with three of their own strains and found the lowest carotene concentration in the uppermost leaf of the plant in two of the three analyses made. The results are shown in table 5.

TABLE 5.—*Carotene concentration of different leaves from the same plant*

| Strain | Stage of maturity | Position of leaves [1] | Carotene content |
|---|---|---|---|
| | | | *Micrograms per gram* |
| Single hybrid No. 1 | Late silk | 1 and 2 [1] | 547 |
| | | 5 and 6 | 730 |
| | | 9 and 10 | 800 |
| Single hybrid No. 2 | Medium milk | 1 and 2 | 474 |
| | | 5 and 6 | 615 |
| | | 8 and 9 | 559 |
| $R_4$ | Late silk | 2 and 3 | 593 |
| | | 4 and 5 | 663 |
| | | 8 and 9 | 572 |

[1] Counting from top to bottom.

Further analyses were made to determine whether there is a difference in the carotene concentration of the tip and basal portions of the same leaf. The blades of two or three leaves were cut transversely, and the two halves were analyzed. The results, given in table 6, show that the differences were rather slight.

TABLE 6.—*Carotene concentration in tip and basal portions of the same leaf*

| Strain | Stage of maturity | Carotene content | |
|---|---|---|---|
| | | Tip half | Basal half |
| | | *Micrograms per gram* | *Micrograms per gram* |
| Single hybrid No. 1 | Late silk | 613 | 623 |
| Single hybrid No. 2 | Medium milk | 411 | 424 |
| | Early dent | 463 | 460 |
| | Medium dent | 499 | 477 |
| $R_4$ | Very early milk | 458 | 374 |

## EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS ON THE CAROTENE CONTENT OF CORN PLANTS

### STRAIN DIFFERENCES

In 1940, at the early dent stage of maturity the leaf blades of 25 inbred strains were found to range in carotene concentration from 213 to 544 micrograms per gram and the remainder of the plant from 6 to 24. To determine the statistical significance of such strain differences, analysis of variance tests were performed on the results of analyses made on these strains at anthesis and at the medium dent stage of maturity. Table 7 summarizes the results of the tests and shows that the variance between strains is highly significant at both stages of development.

TABLE 7.—*Analysis of variance table to test the significance of strain differences in carotene concentration of leaf blades*

STAGE OF DEVELOPMENT—ANTHESIS

| Variance | Degrees of freedom | Sum of squares | Mean square | $F$ [1] | $F$ (.05) [1] |
|---|---|---|---|---|---|
| Within strains | 25 | 12,214 | 489 | | |
| Between strains | 24 | 560,940 | 23,373 | 47.8 | 1.96 |
| Total | 49 | 573,154 | | | |

STAGE OF DEVELOPMENT—MEDIUM DENT

| | | | | | |
|---|---|---|---|---|---|
| Within strains | 27 | 23,017 | 853 | | |
| Between strains | 26 | 1,032,386 | 39,707 | 46.6 | 1.92 |
| Total | 53 | 1,055,403 | | | |

[1] Ratio of variance.

Calculations of the total carotene, dry weight, and carotene concentration per plant of individual strains harvested at the medium dent stage were also made. The results, which are summarized in table 8, show that the strains differed greatly. Similar ranges were found in the other 3 years in which the same analyses were made.

TABLE 8.—*Carotene and dry weight content of inbred, single, and double hybrid strains of corn at the medium dent stage of maturity in 1940*

| Strains | | Range of carotene per plant | Carotene concentration | | Dry weight per plant |
|---|---|---|---|---|---|
| Type | Number analyzed | | Entire plant | Leaf blades | |
| | | *Milligrams* | *Micrograms per gram* | *Micrograms per gram* | *Grams* |
| Inbred | 27 | 3.4–14.1 | 20.5–75.1 | 167–503 | 114–261 |
| Single hybrid | 18 | 7.1–29.2 | 33.6–73.0 | 246–529 | 172–458 |
| Double hybrid | 30 | 9.7–17.5 | 29.2–76.0 | 210–690 | 205–364 |

STAGE OF MATURITY

In order to learn something of the variation in carotene content of corn plants with age, large plantings of nine strains were made in the summer of 1940, and samples were taken at definite intervals throughout most of the growing season. The results are summarized graphically in figures 2 to 5. Each graph represents average values for the nine strains, except those in figure 2, *A* and *B*, where the values for each strain are shown separately.

The concentration of carotene in the leaves reached a sharp peak at 78 days after planting, when the plants were shedding pollen, but the concentration in the entire plant dropped steadily throughout the period studied. The total carotene content of the entire plant, on the other hand, increased rapidly to a high level at 68 days, and then remained more or less constant until near the end of the season, when it also fell off sharply. The total carotene produced per acre in

comparison with the total yield of silage at different periods of harvest is shown in table 9. It is obvious that large loss of carotene occurs if the harvesting is delayed until the late dent stage.

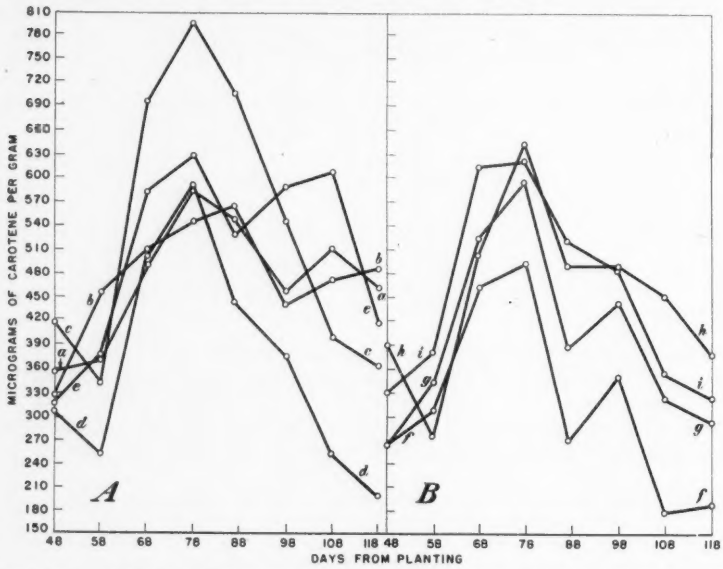


FIGURE 2.—Carotene concentration in the leaf blades of (*A*) five strains of corn grown in 1940 and (*B*) four additional strains grown in 1940: *a*, Iowa single hybrid sweet corn; *b*, Country Gentleman sweet corn; *c*, inbred 153; *d*, inbred 3408; *e*, single hybrid 3 x 26; *f*, inbred 6; *g*, inbred 26; *h*., inbred 3; *i*, inbred R19.

TABLE 9.—*Yield of carotene and silage at different harvests (1940 season)*

| Strain or variety | Harvest [1] | Yield per acre | |
|---|---|---|---|
| | | Carotene | Silage [2] |
| | | *Grams* | *Tons* |
| 525 | First | 206 | 10.8 |
| | Second | 255 | 12.3 |
| | Third | 134 | 12.8 |
| Golden Glow | First | 166 | 11.1 |
| | Second | 166 | 11.3 |
| | Third | 103 | 11.1 |
| 645 | First | 243 | 12.4 |
| | Second | 230 | 14.1 |
| | Third | 93 | 14.2 |
| 570 | First | 174 | 12.3 |
| | Second | 169 | 14.1 |
| | Third | 99 | 14.0 |
| 606 | First | 248 | 14.7 |
| | Second | 126 | 13.2 |
| | Third | 99 | 15.2 |
| Funk 235 | First | 248 | 13.8 |
| | Second | 166 | 14.9 |
| | Third | 111 | 15.7 |
| Average of all strains and varieties | First | 214 | 12.5 |
| | Second | 185 | 13.3 |
| | Third | 107 | 13.8 |

[1] The first harvest was made at the early dent stage, the second at the medium dent stage, and the third at the late dent stage.
[2] The yields of silage are calculated to a 70-percent moisture basis.

As is well known, strains of corn can be classified according to the number of days required to reach a specific stage of development. When a group of inbred strains was compared on this basis, it was found that with few exceptions those which required the longest time to reach anthesis averaged the most carotene (concentration of leaves and content of the entire plant) and those requiring the shortest period had the least. Those intermediate in carotene content were also intermediate in their growth period requirements. The data are summarized in table 10. It is questionable whether the higher

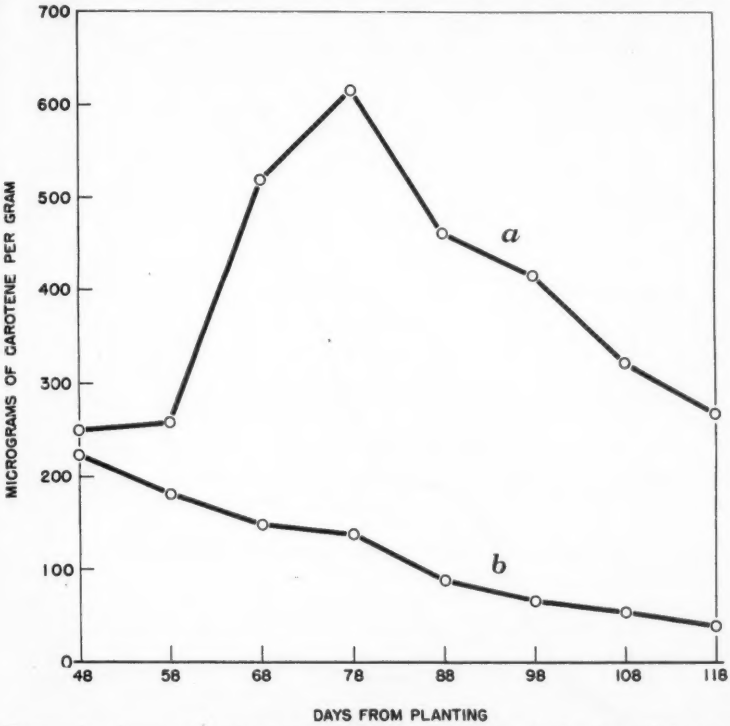


FIGURE 3.—Average carotene concentration of the nine strains for which data are shown in figure 2, *A* and *B*: *a*, Leaf blades; *b*, entire plant.

carotene content of the slower growing strains is the result of the longer growth period or is due to inherently higher carotene synthesis. The former seems more probable since the carotene concentration of corn leaves increases at a faster rate than the development of the leaf itself. Hence the carotene content of two strains having the same capacity for carotene synthesis would probably be higher in the one requiring the longer growth period to reach anthesis.

That strains differ in their inherent capacity for carotene formation seems fairly certain also, for when several strains which reached the same stage of development at the same time were compared

(table 11), it was found that they differed significantly in carotene content. Here the influence of external factors in producing strain differences was kept at a minimum.

Table 10 also shows the decrease in carotene during the interval from anthesis to the early dent stage. It will be noted that those

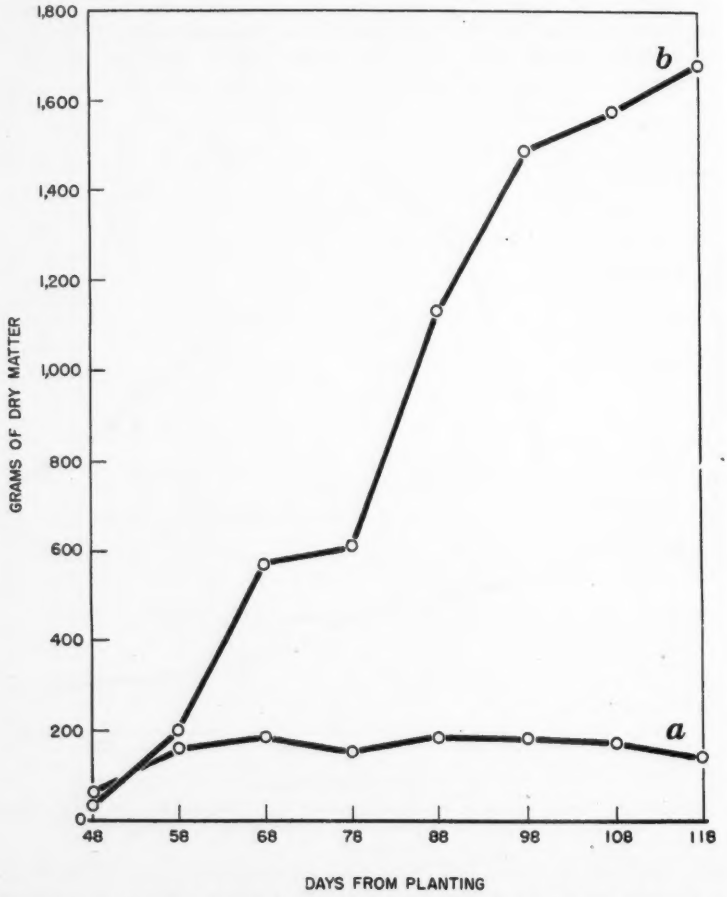


FIGURE 4.—Average dry weight per eight plants of the nine strains for which data are shown in figure 2, *A* and *B*: *a*, Leaf blades; *b*, remainder of plant.

strains requiring the longest growth period lost the most carotene. Whether this loss is correlated with the production of some specific substance in the plant has not been determined. Certainly the decrease in carotene content is not proportional to the dry weight increase of a strain during the same growing period (table 12).

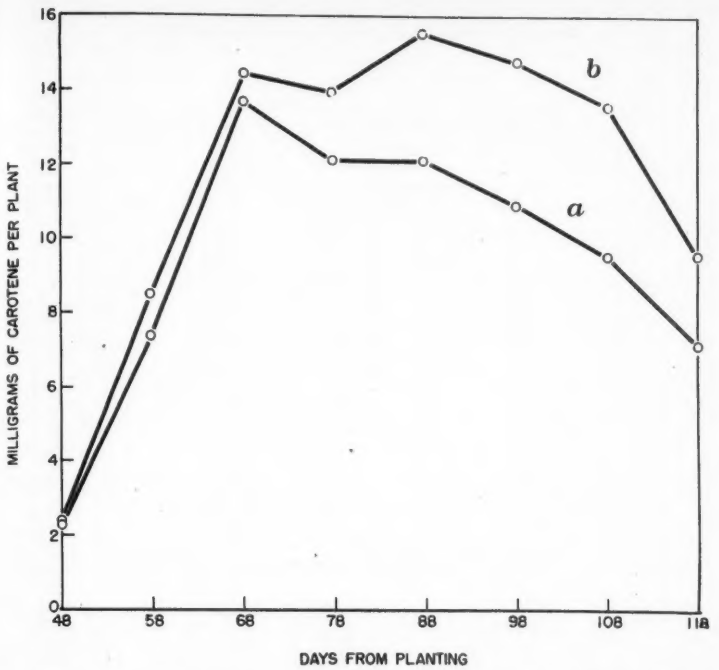


FIGURE 5.—Average total carotene content per plant of the nine strains for which data are shown in figure 2, *A* and *B*: *a*, Leaf blades; *b*, entire plant.

TABLE 10.—*Effect of length of growth period on the carotene concentration of corn*

| Interval from planting to anthesis (days) | Strains averaged [1] | Carotene in leaf blades at anthesis | | Interval from anthesis to early dent stage | Strains averaged [1] | Loss of carotene from leaf blades | |
|---|---|---|---|---|---|---|---|
| | | Range | Average | | | Range | Average |
| | | *Micrograms per gram* | *Micrograms per gram* | *Days* | | *Micrograms per gram* | *Micrograms per gram* |
| 73–77 | {25; $M_{13}$; S. R. $M_{13}$; 9; S. R. 3. | 468–565 | 523 | 26–33 | {9; S. R. 23; 25; $M_{13}$; 23. | 105–271 | 177 |
| 79–82 | {S. R. 26; 8–29; 14; 375; 4362; 23; S. R. 23; $R_3$; A; 38. | 535–736 | 620 | 35–40 | {S. R. $M_{13}$; S. R. 3; S. R. 26; 14; $R_3$; A; $A_{48}$; 32; 374; 90; Hy; $R_4$; 4391; 8; 4415. | 128–477 | 281 |
| 84 | {$A_{48}$; 32; $WF_9$; 374; 90; Tr. | 535–701 | 619 | 42–51 | {8–29; 375; 4362; 38; $WF_9$; Tr; $L317B_2$. | 149–574 | 306 |
| 89 | {Hy; $R_4$; $L317B_2$; 4391; 8; 4415. | 618–900 | 786 | | | | |

[1] "S. R." denotes sun-red strain.

TABLE 11.—*Differences in carotene formation among strains that reached the same stage of development at the same time (season 1940)*

| Strain | Anthesis | | Strain | Early dent stage | |
|---|---|---|---|---|---|
| | Interval from planting to harvest | Carotene concentration of leaf blades | | Interval from planting to harvest | Carotene concentration of leaf blades |
| | *Days* | *Micrograms per gram* | | *Days* | *Micrograms per gram* |
| Hy | 89 | 618, 636 | 32 | 121 | 362, 372 |
| $R_4$ | 89 | 837, 835 | $A_{48}$ | 121 | 530, 460 |
| 8 | 89 | 729, 751 | 4362 | 121 | 320, 330 |
| 4391 | 89 | 900, 828 | 374 | 122 | 378, 414 |
| $L317B_2$ | 89 | 854, 890 | 90 | 122 | 396, 408 |
| 4415 | 89 | 792, 760 | $R_3$ | 122 | 267, 271 |
| | | | 8–29 | 124 | 522, 544 |
| | | | 8 | 124 | 520, 484 |

TABLE 12.—*Decrease in total carotene content of leaves and increase in dry weight of entire plant from pollen-shedding stage to medium dent stage of maturity*

| Inbred strain | Carotene decrease per plant | Dry weight increase per plant | Inbred strain | Carotene decrease per plant | Dry weight increase per plant |
|---|---|---|---|---|---|
| | *Milligrams* | *Grams* | | *Milligrams* | *Grams* |
| 25 | 2.29 | 62 | $A_{48}$ | 7.30 | 95 |
| $M_{13}$ | 3.40 | 65 | 32 | 5.36 | 121 |
| Sun-red $M_{13}$ | −.63 | 55 | $WF_9$ | 2.15 | 147 |
| 9 | .81 | 87 | 374 | 7.88 | 87 |
| Sun-red 3 | 1.29 | 152 | 90 | .84 | 74 |
| 8–29 | 4.42 | 69 | Tr | 9.18 | 127 |
| 14 | 6.79 | 89 | Hy | 9.14 | 111 |
| 375 | −2.15 | 130 | $R_4$ | 10.30 | 110 |
| 4362 | 5.21 | 135 | $L317B_2$ | 9.03 | 150 |
| 23 | 6.49 | 94 | 4391 | 8.95 | 96 |
| Sun-red 23 | 5.15 | 101 | 8 | 6.37 | 106 |
| $R_3$ | 5.65 | 54 | 4415 | 15.00 | 66 |
| A | 5.48 | 65 | Sun-red 26 | 2.74 | 86 |
| 38 | 9.75 | 102 | | | |

## SEASON

The carotene concentration of the leaves and of the entire plant of a strain and the total carotene content of the plant differed considerably when harvests were made at the same stage of maturity in two successive years (table 13). To test the significance of these differences the results of analyses of the carotene concentration of the leaf blades of five strains at the early, medium, and late dent stages in 1940 and 1941 were subjected to an analysis of variance test. Table 14 shows that the variance between years is highly significant. A similar test was also applied to eight additional strains analyzed at the flowering and medium dent stages for the years 1940 and 1941, with the results shown in table 15. Again it is found that the carotene concentration of the leaf blades differs significantly from year to year. Tables 14 and 15 also show that of the three variables, strain, year, and stage of development, the last named affects the carotene concentration of a plant most. The other two factors are about equal in their effect.

TABLE 13.—*Carotene concentration of corn plants grown in 1940 and 1941 and harvested at the medium dent stage of maturity*

| Hybrid or variety | Carotene concentration | | | | Total carotene per plant | |
|---|---|---|---|---|---|---|
| | Leaf blades | | Entire plant | | | |
| | 1940 [1] | 1941 [1] | 1940 | 1941 | 1940 | 1941 |
| | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Milligrams* | *Milligrams* |
| 525 | 615; 607 | 479; 498 | 76.0 | 59.5 | 17.0 | 15.2 |
| 526 | 480; 536 | 405; 404 | 47.7 | 57.1 | 11.2 | 13.6 |
| 531 | 622; 605 | 445; 499 | 69.0 | 39.5 | 15.7 | 10.4 |
| 606 | 459; 459 | 458; 387 | 58.8 | 54.8 | 16.1 | 13.0 |
| 572 | 690; 572 | 354; 413 | 74.4 | 51.0 | 17.5 | 13.5 |
| 603 | 493; 498 | 340; 332 | 60.0 | 35.8 | 14.6 | 11.6 |
| Golden Glow | 404; 465 | 381; 434 | 53.5 | 46.4 | 10.9 | 11.8 |
| Murdock | 403; 405 | 370; 349 | 50.4 | 23.5 | 12.6 | 5.4 |
| 645 | 544; 580 | 489; 440 | 60.5 | 55.9 | 15.4 | 16.6 |
| 625 | 410; 454 | 428; 405 | 49.0 | 36.0 | 17.2 | 10.6 |
| 640 | 406; 330 | 440; 422 | 45.5 | 73.1 | 13.8 | 16.9 |
| 648 | 400; 368 | 361; 349 | 44.5 | 35.6 | 13.2 | 8.8 |
| 675 | 403; 412 | 304; 311 | 43.9 | 29.8 | 10.0 | 9.9 |
| 676 | 392; 439 | 341; 350 | 45.6 | 40.2 | 13.6 | 10.8 |
| 680 | 360; 327 | 404; 402 | 32.7 | 50.4 | 10.8 | 14.9 |
| 697 | 376; 325 | 388; 431 | 38.3 | 32.7 | 13.9 | 10.8 |
| Pioneer 322 | 290; 340 | 338; 364 | 30.6 | 35.2 | 9.8 | 9.2 |
| 696 | 398; 408 | 285; 288 | 34.9 | 35.0 | 10.5 | 10.9 |
| DeKalb 404A | 458; 435 | 300; 322 | 43.9 | 39.9 | 14.7 | 10.5 |
| 690 | 382; 347 | 300; 292 | 42.4 | 36.2 | 12.2 | 13.1 |
| 695 | 353; 339 | 358; 358 | 45.0 | 42.0 | 10.4 | 12.0 |
| Iowa 13 | 410; 413 | 386; 488 | 37.8 | 44.1 | 11.0 | 13.6 |
| Funk 235 | 330; 387 | 480; 503 | 40.5 | 50.2 | 12.1 | 12.7 |

[1] Each pair of figures represents the results of duplicate analyses.

TABLE 14.—*Analysis of variance table showing the significance of seasonal variation in carotene content of corn plants harvested at the same stage of maturity, 1940 and 1941*

| Variance | Degrees of freedom | Sums of squares | Mean square | *F* [1] | *F* (0.05) [1] |
|---|---|---|---|---|---|
| Within strains | 30 | 21,308 | 710 | 1 | |
| Between strains | 4 | 89,699 | 22,425 | 31.6 | 2.69 |
| Between harvests | 2 | 282,403 | 141,202 | 198.9 | 3.32 |
| Between years | 1 | 41,870 | 41,870 | 59.0 | 4.17 |
| Strain × harvest interaction | 8 | 49,739 | 6,217 | 8.8 | 2.27 |
| Strain × year interaction | 4 | 58,274 | 14,569 | 20.5 | 2.69 |
| Harvest × year interaction | 2 | 29,908 | 14,954 | 21.0 | 3.32 |
| Strain × harvest × year interaction | 8 | 116,345 | 14,543 | 20.5 | 2.27 |
| Total | 59 | 689,546 | | | |

[1] Ratio of variances.

TABLE 15.—*Analysis of variance table showing the significance of seasonal variation in carotene content of corn plants at flowering and medium dent stages, 1940 and 1941*

| Variance | Degrees of freedom | Sums of squares | Mean square | *F* [1] | *F* (.05)[1] |
|---|---|---|---|---|---|
| Within strains | 32 | 32,672 | 1,021 | 1 | |
| Between strains | 7 | 308,399 | 44,057 | 43.2 | 2.32 |
| Between harvests | 1 | 1,362,764 | 1,362,764 | 1,335.0 | 4.15 |
| Between years | 1 | 26,122 | 26,122 | 25.6 | 4.15 |
| Strain×harvest interaction | 7 | 67,191 | 9,599 | 9.4 | 2.32 |
| Strain×year interaction | 7 | 26,282 | 3,740 | 3.7 | 2.32 |
| Year×harvest interaction | 1 | 7,679 | 7,679 | 7.5 | 4.15 |
| Strain×year×harvest interaction | 7 | 13,914 | 1,988 | 1.9 | 2.32 |
| Total | 63 | 1,845,023 | | | |

[1] Ratio of variances.

### Prevention of Pollination

Pollination was prevented in several strains by covering ear shoots with paper bags from the time of tasseling until pollinated sister plants in the same row were at the desired stage of maturity. The carotene concentration of the leaves of these pollinated and unpollinated plants is shown in table 16. From the results it is evident that the decrease

Table 16.—*Carotene concentration of the entire leaves of pollinated and unpollinated corn plants*

| Strain or variety | Carotene concentration at— | | | |
|---|---|---|---|---|
| | Early dent stage | | Medium dent stage | |
| | Pollinated | Unpollinated | Pollinated | Unpollinated |
| | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* |
| 525 | [1] 51 | [1] 132 | 101 | 108 |
| | 46 | 59 | 110 | 122 |
| 570 | 63 | 77 | 74 | 67 |
| | 96 | 85 | 109 | 103 |
| 645 | 143 | 100 | 111 | 75 |
| | 156 | 91 | 135 | 93 |
| 696 | 95 | 59 | 88 | 51 |
| | 126 | 57 | 111 | 52 |
| Funk hybrid 235 | 100 | 96 | 85 | 59 |
| | 124 | 120 | 120 | 58 |
| Golden Glow | [1] 44 | [1] 42 | 119 | [2] 0 |
| | 54 | 35 | 96 | |

[1] Values given are for entire plant.
[2] These plants were completely lacking in color and were not analyzed.

in carotene concentration of leaves occurs independently of seed formation. Indeed, in some cases the leaf carotene concentration is much lower where pollination was prevented.

### Sugary Character

It was found that the carotene concentration of the leaves of two sweet corn strains did not decrease in the later stages of development as in dent corn strains. This is clearly shown in figure 6. Otherwise the changes in carotene concentration were similar in the leaves of sweet corn and dent corn strains.

### Sun-Red Character

Sun-red strains of corn contain considerable amounts of anthocyanin pigments whereas dilute sun-red strains have little. By crossing with a sun-red strain and then backcrossing to the original parent it is possible to select a sun-red strain which is almost identical in all other respects with the original non-sun-red (dilute) strain. The carotene concentration of the leaves of four such closely related strains were determined and are reported in table 17. It is apparent that the sun-red character has no effect upon the carotene concentration of corn leaves, for sometimes the sun-red strains had more and at other times less carotene per gram of dry leaf tissue than homologous non-sun-red strains.

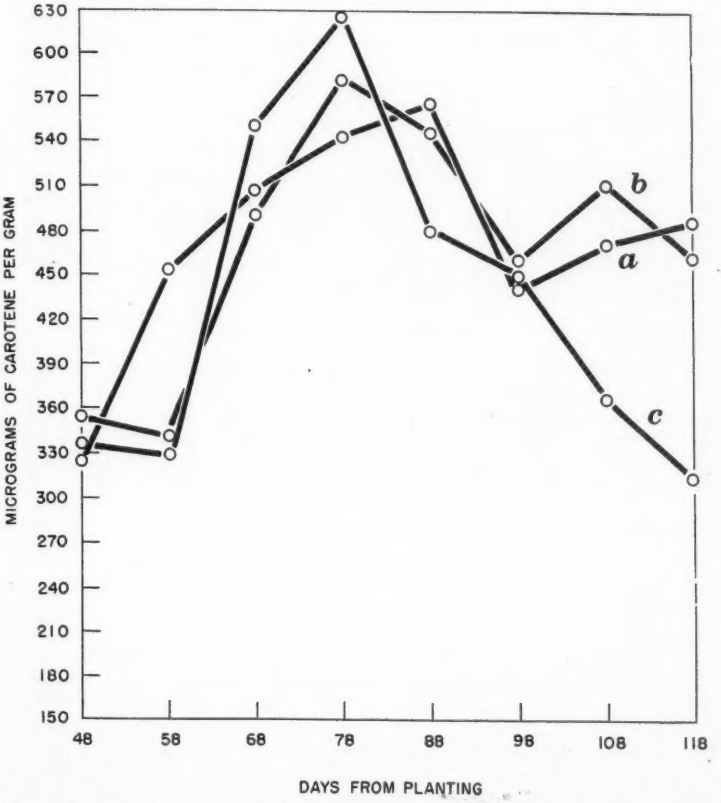


FIGURE 6.—Carotene concentration in the leaf blades of various strains of corn during the 1940 season: *a*, An Iowa single hybrid sweet corn; *b*, Country Gentleman sweet corn; *c*, average concentration of six inbred and one single hybrid dent corn strains.

TABLE 17.—*Carotene concentration of sun-red and non-sun-red strains of corn, 1939 and 1940*

| Strain | Carotene concentration in 1939 [1] at— | | Carotene concentration in 1940 [2] at— | | |
|---|---|---|---|---|---|
| | Early dent stage | Medium dent stage | Anthesis | Early dent stage | Medium dent stage |
| | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* |
| Sun red M 13 | | 144 | 534 | 334 | 503 |
| Non sun red M 13 | | 159 | 508 | 336 | 467 |
| | | 144 | 526 | 368 | 300 |
| | | 127 | 565 | 378 | 344 |
| Sun red 26 | 50 | 50 | 572 | 428 | 378 |
| | 72 | 50 | 581 | 399 | 378 |
| Non sun red 26 | 46 | 77 | 582 | | 283 |
| | 60 | 84 | 602 | | 296 |
| Sun red 3 | 127 | 70 | 532 | 405 | 375 |
| | 129 | 60 | | 403 | 365 |
| Non sun red 3 | | 65 | 637 | | 366 |
| | | 73 | 640 | | 386 |
| Sun red 23 | 42 | 50 | 625 | 413 | 346 |
| | 67 | 51 | 641 | 428 | 306 |
| Non sun red 23 | 74 | 85 | 664 | 433 | 288 |
| | 113 | 98 | 736 | 425 | 404 |

[1] Analyses were made on entire leaf (sheath and blade).
[2] Analyses were made on leaf blade only.

## NUMBER OF PLANTS PER HILL

The effect of the number of plants per hill on the carotene content of corn is an important practical question. Table 18 shows the concentration of carotene in the leaf blades and the entire plant and the total carotene content per hill when two, four, and six plants were grown in hills 3 feet apart. The six-plant hills produced over twice as much carotene as those of only two plants.

TABLE 18.—*Effect of number of corn plants per hill on carotene concentration*[1]

| Strain No. | Plants per hill | Carotene concentration— | | Total dry weight per hill of— | | Total carotene per hill |
|---|---|---|---|---|---|---|
| | | Leaf blades | Entire plant | Leaf blades | Remainder of plant | |
| | | *Micrograms per gram* | *Micrograms per gram* | *Grams* | *Grams* | *Milligrams* |
| 625 | 2 | 465 | 40 | 51 | 825 | 35.0 |
| | | 504 | | | | |
| | 4 | 400 | 42 | 111 | 1220 | 55.9 |
| | | 363 | | | | |
| | 6 | 388 | 55 | 151 | 1330 | 81.5 |
| | | 443 | | | | |
| 696 | 2 | 378 | 33 | 69 | 885 | 31.5 |
| | | 333 | | | | |
| | 4 | 267 | 30 | 108 | 1190 | 38.9 |
| | | 291 | | | | |
| | 6 | 349 | 35 | 158 | 1660 | 63.6 |
| | | 327 | | | | |

[1] Analyses were made on plants at the medium dent stage of development.

## RELATION OF CAROTENE CONTENT OF INBRED AND HYBRID STRAINS

It is desirable to know whether one can predict the carotene content of a hybrid from that of its inbred parents. Table 19 presents data which give information on this question. It is evident that a prediction of a hybrid's carotene concentration can be made with only a slight degree of accuracy from the data on the parent inbred strains.

It will also be noted from table 19 that hybrids had a much higher average carotene concentration than inbreds at the medium dent stage. It is not certain whether this is a reflection of the practice of selecting hybrids retaining much of their color at the later stages of maturity or whether it is a definite result of hybridization. Certainly the higher content per plant is largely traceable to the latter factor since a hybrid has a greater weight of leaves per plant than either of its inbred parents.

TABLE 19.—*Carotene content of hybrids and their inbred parents*

| Strains | Carotene concentration of— | | | | | | Total carotene content per plant | |
|---|---|---|---|---|---|---|---|---|
| | Leaves | | | | Entire plant | | | |
| | Hybrids [1] | Inbred parents [1] | Hybrids [2] | Inbred parents [2] | Hybrids [2] | Inbred parents [2] | Hybrids [2] | Inbred parents [2] |
| | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Micrograms per gram* | *Milligrams* | *Milligrams* |
| $K_{19} \times M_{13}$ | 586 | [3] 546; 617 | 415 | [3] 352; 322 | 73.0 | [3] 51.0; 42.5 | 12.8 | [3] 9.7; 5.2 |
| 23×26 | 559 | 700; 592 | 385 | 346; 290 | 50.5 | 44.1; 39.4 | 14.1 | 7.4; 6.8 |
| 153×8 | 606 | 792; 740 | 308 | 400; 398 | 53.4 | 65.9; 52.5 | 11.2 | 9.2; 10.9 |
| $22 \times R_3$ | 455 | 776; 632 | 293 | 286; 258 | 46.6 | 35.5; 38.2 | 10.2 | 7.5; 5.2 |
| $M_{13} \times R_3$ | 615 | 546; 632 | 331 | 322; 258 | 48.2 | 42.5; 38.2 | 8.7 | 5.2; 5.2 |
| $153 \times M_{13}$ | 625 | 792; 546 | 461 | 400; 322 | 57.9 | 65.9; 42.5 | 11.4 | 9.2; 5.2 |
| $K_{19} \times 153$ | 608 | 792; 617 | 357 | 352; 400 | 48.4 | 51.0; 65.9 | 8.3 | 9.7; 9.2 |
| $3 \times R_3$ | 623 | 639; 632 | 341 | 376; 258 | 55.0 | 38.0; 38.2 | 11.5 | 8.6; 5.2 |
| $374 \times A_{48}$ | 679 | 683; 661 | 429 | 299; 361 | 47.5 | 30.4; 52.1 | 13.9 | 6.0; 10.2 |
| 3×26 | 624 | 639; 592 | 468 | 376; 290 | 50.4 | 38.0; 39.4 | 16.1 | 8.6; 6.8 |
| 38×22 | 553 | 552; 776 | 304 | 180; 286 | 38.5 | 20.5; 35.5 | 16.1 | 4.1; 7.5 |
| $375 \times R_3$ | 565 | 557; 632 | 253 | 377; 258 | 33.6 | 56.8; 38.2 | 7.1 | 9.3; 5.2 |
| $WF_9 \times R_3$ | 546 | 553; 632 | 403 | 255; 258 | 54.5 | 43.6; 38.2 | 17.4 | 10.2; 5.2 |
| $90 \times WF_9$ | 589 | 596; 553 | 522 | 382; 255 | 66.8 | 73.3; 43.6 | 23.4 | 10.6; 10.2 |
| 22×Tr | 665 | 776; 622 | 483 | 286; 349 | 56.6 | 35.5; 53.9 | 25.9 | 7.5; 14.1 |
| $8\text{-}29 \times A_{48}$ | 805 | 719; 661 | 506 | 383; 361 | 72.4 | 47.4; 52.1 | 27.4 | 5.9; 10.2 |
| $WF_9 \times Hy$ | 674 | 553; 627 | 374 | 255; 235 | 47.9 | 43.6; 29.6 | 19.2 | 10.2; 6.8 |
| $8\text{-}29 \times Tr$ | 789 | 719; 622 | 500 | 383; 349 | 72.2 | 47.4; 53.9 | 29.2 | 5.9; 14.1 |

[1] Plants were analyzed at the pollen-shedding stage of development.
[2] Plants were analyzed at the medium dent stage of development.
[3] The first figure in this column refers to the first inbred strain given in column 1; the second figure refers to the other inbred parent.

## DISCUSSION

It is evident that a large variety of both hereditary and environmental factors influence the quantity of carotene produced by a particular corn plant. Other things being equal, the total yield of the pigment depends primarily on the stage of maturity at which the crop is harvested. It appears that the optimum time of harvest with respect both to total dry weight and total carotene comes for most strains at the medium dent stage of development, while most of the plants are still reasonably green and succulent. Delay beyond this time results in a large loss of carotene without much compensating increase in total yield of silage (table 9). It may well be also that the nutritive value of the crop with respect to other labile components is higher while the plants are still green.

Since carotenoid pigments normally accumulate in the ear after pollination, it was thought that the great drop in the carotene concentration in the leaves, usually observed following the pollen-shedding stage, might be avoided if pollination were prevented. Such, however, was not the case. The drop in carotene in these experiments was as great or even greater than when pollination was allowed to proceed normally.

At a given stage of maturity most strains were found to differ widely in successive years. The most striking illustration of this fact was provided by the analytical results secured on plants grown in 1939, which on the average contained only about one-half as much carotene as those grown in 1938. The 1940 results were intermediate between these two. Since 1939 was a season of relatively low rainfall at Madison (9.76 inches from May 1 to October 1 vs. 26.1 inches in 1938 and 18.7 in 1940) there is a strong indication that a liberal moisture supply favors high carotene production.

Another environmental factor, namely, the number of plants grown per hill, also was found to have a considerable influence, at least on the total amount of carotene produced on a given area of land (table 18). Although no attempt was made in this work to study such variables as temperature, light intensity, soil fertility, and the like, there is little doubt that these factors likewise have their effect.

Genetic make-up also must be credited with a considerable influence on the carotene content of corn plants. In general, inbred lines, as might be expected, were found to elaborate less carotene than hybrids. Those strains which required a longer time to reach anthesis showed a higher pigment content than more rapidly developing strains. Although the various strains did not remain in the same relative order at the same stage of maturity in successive years, about 75 percent of them remained in the same relation to the average for the entire group at each harvest (i. e., either above or below the average). Furthermore, a few hybrid strains, notably 525, 645, and 531, were found to be consistently among the high carotene groups for 1939, 1940, and 1941. Efforts to correlate the carotene content of various hybrids with that of their inbred parents, however, were not particularly successful (table 19). Likewise the presence or absence of the sun-red gene has almost no effect. In general it seems that the amount of genetic variability with reference to this constituent of the corn plant is not sufficient to justify efforts, for the present at least, towards breeding corn for higher carotene content.

## SUMMARY

The concentration of carotene in corn plants is 20 to 50 times higher in the leaf blades than in the remainder of the plant. Stalks, ears, and husks all have about the same concentration, at least in the early dent stage of growth.

Concentration of carotene in the leaves reaches a peak at the pollen-shedding stage and then declines rapidly. Prevention of pollination by bagging the ear shoot did not prevent this drop. The total carotene content of the plant, however, remains relatively constant until near the end of the growing season, when it also falls sharply.

The optimum period for harvesting with respect to total yield of both carotene and silage comes for most strains at the medium dent stage of maturity.

Significant differences in carotene content between stocks, both inbred and hybrid, exist, but are small relative to those associated with stage of development of the plant and with seasonal factors.

Data on two strains suggest that sweet corns may retain a relatively high carotene content as the season advances.

The sun-red gene does not influence the carotene content of corn plants.

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# LEAF ELONGATION AND FRUIT GROWTH OF THE DEGLET NOOR DATE IN RELATION TO SOIL-MOISTURE DEFICIENCY [1]

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## INTRODUCTION

Successful date growing in the United States is confined largely to the desert valleys of California and Arizona, where air temperatures in summer fluctuate from minima of 70° to 90° F. to maxima of 95° to 116° or more. With these high temperatures, together with low relative humidity, soil moisture is rapidly depleted by the date palm (*Phoenix dactylifera* L.) and for successful date production it must be frequently replenished by irrigation. Since the palm does not exhibit any easily observed symptom of water shortage, such as leaf wilting, it frequently suffers from soil-moisture deficiency for many weeks without the grower's knowing it.

For this reason an investigation was begun in 1938 at the United States Date Garden, near Indio, Calif., to find (1) a simple method of detecting when a date palm is being influenced by a deficiency in soil moisture and (2) the extent to which a temporary deficiency in soil moisture may influence fruit size and quality. The systematic measurement of elongation of recently emerged leaves, originally used by Mason (*6*) [3] and later tested to a limited extent by Pillsbury (*10*), was carefully studied. The details of the technique which was finally developed, together with a discussion of factors other than soil moisture that affect the rate of leaf elongation, have already been reported (*2*).

The effects of soil-moisture deficiency during the summer upon fruit development in relation to rate of leaf elongation were determined in four experiments, from 1939 to 1941, each experiment in a different planting of bearing Deglet Noor palms. The difficulties involved in obtaining suitable palms with a satisfactory supply and distribution of irrigation water made replications of treatments in any one experiment impracticable. Soil-moisture deficiency was obtained in experimental plots by omitting regular irrigations for a single period of 5 to 10 weeks, but applying at all other times the same irrigation (3 to 9 acre-inches every 10 days) as in the control. In all experiments plot A was the frequently irrigated control and plots B, C, and D had soil-moisture deficiencies in late June and early July,

[1] Received for publication July 26, 1944.
[2] The writers appreciate the cooperation of H. V. Cavanagh and Kenneth Peck, who kindly furnished parts of their commercial gardens for field plots.
[3] Italic numbers in parentheses refer to Literature Cited, p. 200.

in late July and early August, and in late August and early September, respectively. The measurements of average fruit growth and average rate of leaf elongation for each plot were based upon 3 to 6 palms. Fruit growth was measured by determining fresh and dry weights per fruit in weekly collections of 60 to 96 fruits per plot. In order that all fruits might be relatively uniform in development they were selected only from bunches known to have been pollinated within a 14-day period in the spring.

## RATE OF PALM LEAF ELONGATION IN RELATION TO SOIL MOISTURE

To substantiate preliminary results obtained in 1938 (7), indicating that the rate of leaf elongation is a sensitive index of the extent of water deficit in the palm, rate of leaf elongation in relation to soil

FIGURE 1.—Ten-year-old Deglet Noor date palms in plot C of the Cavanagh garden on June 4, 1940, being irrigated at the rate of 0.5 cubic foot per second.

moisture was carefully studied in four plots in the Cavanagh garden in 1940. Vigorous 10-year-old palms irrigated by flooding (fig. 1) were used, and one guard row of palms was left between the plots.

The soil, apparently an Indio very fine sand, with no uniform horizons but with strata of silt of varying thicknesses at different depths below the soil surface, is representative of a large proportion of the better date soils. Approximately 3 to 4 acre-inches of water was applied at each irrigation during May and early June and 7 to 9 inches thereafter. Some of this water moved laterally into the unirrigated middle. The average soil moisture per plot was based on a sample of the composite of all soil at each foot depth removed by a soil tube at 12 locations per plot, usually taken the day before and the day after each irrigation. The rate of leaf elongation was based upon measurement of 2 leaves every 2 or 3 days on each of 6 palms per plot.

LEAF ELONGATION WITH FREQUENT IRRIGATION

The frequent irrigation of plot A (control) was representative of commercial plantings using as much as 12 to 14 acre-feet of water annually. After June 25 each irrigation increased the percentage of soil moisture in each of the top 5 feet to more than twice the moisture-equivalent values (table 1). The average percentage moisture content was usually still above the moisture equivalent the day before the next irrigation. The average rate of moisture decrease between irrigations for the top 6 feet, expressed as volume of water, averaged 0.121 acre-inch per day per foot depth of soil, or a total of 7.26 acre-inches in the top 6 feet during 10 days.

The rate of leaf elongation (fig. 2) in plot A between May 10 and October 17 fluctuated between 3.6 and 5.3 cm. per day. The temporarily low rates of elongation during the summer coincided with hot, dry weather and the temporarily high rates with cooler or relatively humid weather. The minima in rates in plot A during hot, dry weather are interpreted as indicating slight water deficits in the palms when transpiration from the leaves exceeded the water supplied by the roots. Minimum rates were frequently followed immediately by relatively high rates, probably resulting from resumption of turgor of enlarging leaf tissue, augmenting normal growth.

TABLE 1.—*Soil-moisture constants and volume weight of soil in plot A (control), Cavanagh garden, 1940*

| Soil depth (feet) | Moisture equivalent [1] | Wilting range [2] | | Volume weight [3] |
|---|---|---|---|---|
| | | First permanent wilting point | Ultimate wilting point | |
| | *Percent* | *Percent* | *Percent* | *Grams per cubic centimeter* |
| 0–1 | 7.1 | 3.0 | 2.3 | 1.43 |
| 1–2 | 5.4 | 2.6 | 1.9 | 1.36 |
| 2–3 | 6.7 | 3.0 | 1.9 | 1.39 |
| 3–4 | 4.9 | 2.2 | 1.7 | 1.39 |
| 4–5 | 4.0 | 2.1 | 1.5 | 1.30 |
| 5–6 | 7.0 | 2.7 | 2.2 | 1.22 |

[1] Determined by J. R. Furr and J. O. Reeve.
[2] Determined by Furr and Reeve by the method which they have recently described (*4*).
[3] Volume weight (also called apparent specific gravity) is dry weight of soil per unit volume of soil in the field.

Immediately after irrigation of plot A on May 23, June 14, July 23, and September 17, the rate of leaf elongation became appreciably greater than just before irrigation. Similar increases were observed by Pillsbury (*9*) and Moore and Aldrich (*7*). Apparently just before irrigation the rate of movement of moisture from the soil into the palm was frequently not sufficient to prevent a slight to moderate water deficit in the palm, but immediately after irrigation the increased water content of the soil in contact with the roots accelerated the rate of water movement into the palm to such an extent that the growth or turgor of cells active in leaf growth was increased. However, for comparison with the appreciable water deficits of palms in plots B, C, and D resulting from the omission of irrigation, the palms in plot A are considered a satisfactory control.

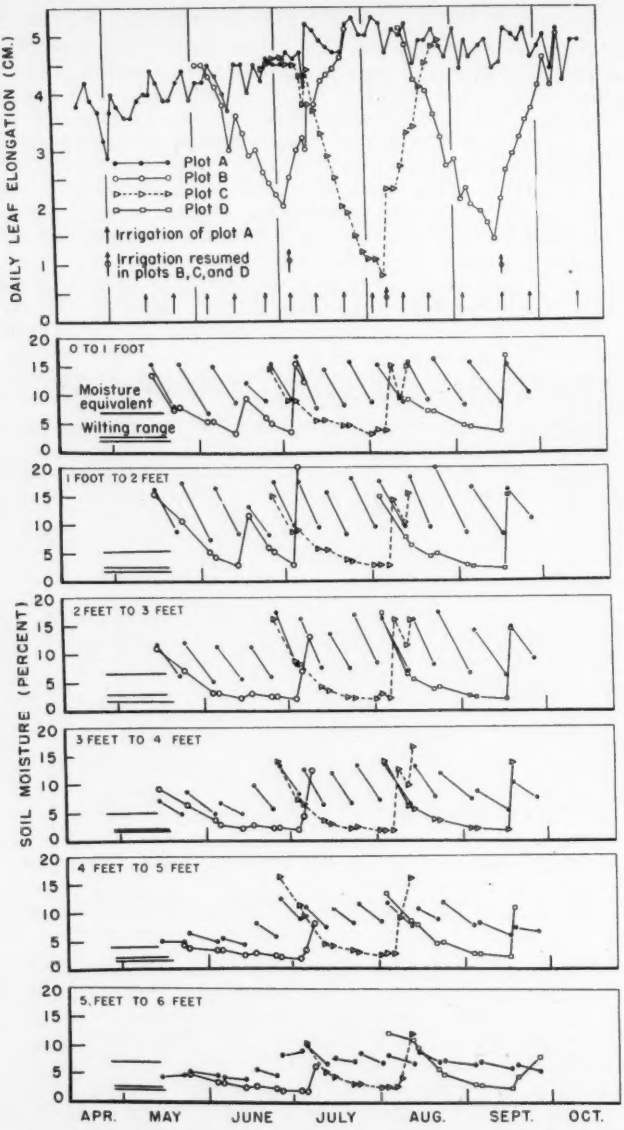


FIGURE 2.—Relation of rate of leaf elongation to average soil-moisture percentages, just after and just before irrigations in plot A (control), and in plots B, C, and D when appreciable water deficits in the date palm were indicated. Plot A was irrigated about every 10 days. In plot B irrigations were omitted from May 14 to July 4, in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The top 2 feet of plot B was accidentally wet on June 14.

### LEAF ELONGATION IN RELATION TO SOIL-MOISTURE DEFICIENCY

As stated previously, irrigations were omitted in plot B from May 14 to July 4 (except that the top 2 feet was accidentally wet on June 14), in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The rate of leaf elongation in plots B, C, and D became slower than that in plot A (fig. 2) when the average soil-moisture percentage decreased below the moisture equivalent and approached the wilting range (*4*) (as determined for the soil in plot A). In general, as the rate of soil-moisture decrease became slower the rate of leaf elongation in plots B, C, and D became progressively lower than that in plot A. By the time the curves for soil moisture had become nearly horizontal, indicating that the soil moisture was in the wilting range and that moisture extraction by the roots was extremely slow, the rate of leaf elongation had decreased below 2 cm. a day. Probably at such times most of the soil moisture that was available to the palm was at depths below 6 feet. These results, together with those obtained earlier (*7*), indicate that a prolonged decrease in rate of leaf elongation below that of adjacent adequately irrigated palms is evidence of soil-moisture deficiency causing an appreciable water deficit in the palm. Wadsworth (*11*) obtained somewhat similar results with sugarcane plants.

After the resumption of irrigation in plots B, C, and D the rate of leaf elongation increased, but not to within 0.5 cm. per day of that in plot A until about 2 weeks had elapsed. During these 2 weeks soil moisture must have been available to the roots, so either a delay in water movement into the roots and from there to the crown of the palm or a delay in resumption of division or enlargement of cells at the base of each growing leaf must have been retarding leaf elongation. Such influences retarding leaf elongation will be considered as a part of the appreciable water deficit in the palm resulting from soil-moisture deficiency. Thus a period of appreciable water deficit will be considered as extending from the time when reduced rate of leaf elongation is first detected to the time when the rate is again within 0.5 cm. per day of that of adjacent adequately irrigated palms.

The data relating rate of leaf elongation to soil moisture indicate that equivalent soil-moisture deficiencies (as measured by rate of soil-moisture decrease near or in the wilting range) in late summer and in midsummer, respectively, caused about the same reduction in rate of leaf elongation. Therefore, in experiments where soil moisture was not measured, it has been assumed that equivalent reductions in rate of leaf elongation (as compared with that in the control plots) indicated equivalent soil-moisture deficiencies.

## EFFECTS OF APPRECIABLE WATER DEFICIT IN THE PALM

### FRUIT DEVELOPMENT

To understand the influence upon fruit development of soil-moisture deficiency at different times during the summer, it was necessary to determine the curves for both fresh- and dry-weight increases of the fruit. Such weight curves were satisfactorily obtained in the previously described Cavanagh plots in 1940, in similar plots in the Cavanagh garden in 1939, in two plots in the Peck garden in 1940, and in somewhat similar plots at the United States Date Garden in 1941. Since the observed effects of appreciable water deficit in the palm

upon the fresh and dry weights of the fruit were essentially the same in each of the experiments, only the curves for the 1940 Cavanagh plots, for which soil-moisture data have already been given, are presented (fig. 3).

The curves in plot A show characteristic changes in the fresh and dry weights of the fruits throughout their growth period. Long (*5*), who studied the anatomy of fruits from the United States Date Garden experiment in 1941, found the relatively slow increase in fresh weight in late April and early May to be characterized by rapid cell division throughout the fruit and by rapid cell enlargement in the apical and median parts; the rapid fresh-weight increase during June and early July to be due almost entirely to cell enlargement, with cell division only in the basal part of the fruit; and the declining fresh-weight increase during late July and early August to be the result of cell enlargement only in the basal part of the fruit. The decrease in fresh weight per fruit during September is the result of a characteristic decrease in water content of Deglet Noor fruit just before final ripening. The rapid increase in dry weight during August and September is, according to Rygg,[4] largely the result of sucrose accumulation in the fruit.

The magnitude and duration of the appreciable water deficits in the palm are indicated (fig. 3) by the extent and length of time that the rate of leaf elongation in other plots is below that in plot A. When the rate of leaf elongation in plots B and C, respectively, dropped more than 0.5 cm. per day below that in plot A, the rate of increase in fresh weight of fruits in plots B and C almost immediately dropped below that of fruits in plot A. The rate of dry-weight increase, however, was not immediately reduced proportionally so much as the rate of fresh-weight increase, with the result that the percentage water content of the fruit (data not given) was temporarily reduced. After the resumption of regular irrigation in plots B and C the fruit remained smaller than in plot A, indicating that the reduced increase in fresh weight during the period of appreciable water deficits in the palm must have been largely due to a restriction in the total number or size of cells and not just to a reduction in water content of the fruit.

Delayed effects of the appreciable water deficit in the palm in plots B and C occurred in late summer, after the resumption of irrigation had resulted in about the same rate of leaf elongation in plots B and C as in plot A. The principal delayed effect was the slower rate of dry-weight increase of the fruit in plots B and C than in plot A during late August or early September. This could have been due to the effects of the appreciable water deficit in the palm in reducing the number or size of cells in which the dry matter could accumulate, in reducing the stored carbohydrates (*1*) available for this dry-matter accumulation, in reducing the efficiency of the vascular bundles for carbohydrate transport, or in modifying the enzymatic or protoplasmic nature of the cells. Associated with the slower rate of dry-weight increase in plots B and C was an approximately 2 weeks' earlier softening of the fruits than in plot A.

An additional delayed effect of appreciable water deficits in the palm in midsummer was observed in plot C in the 1940 experiments

[4] RYGG, G. L. COMPOSITIONAL CHANGES IN THE DATE FRUIT DURING GROWTH AND RIPENING. U. S. Dept. Agr. Tech. Bul. 910.

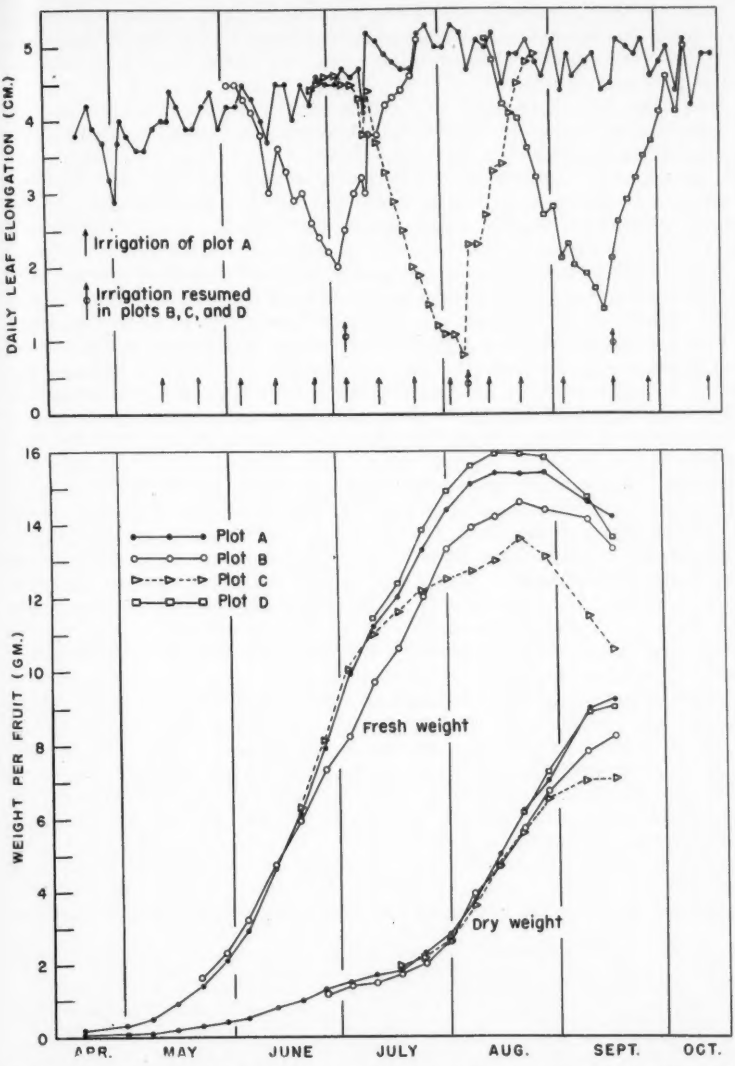


FIGURE 3.—Fresh and dry weights per fruit in relation to appreciable water deficits in the date palm as indicated by rate of leaf elongation, Cavanagh garden, 1940. (Complete curves are presented for plot A, control, but curves for plots B, C, and D are presented only when soil-moisture deficiency caused measurable deviations from curves for plot A.) In plot B irrigation was omitted from May 14 to July 4, in plot C from June 26 to August 7, and in plot D from August 3 to September 17. The top 2 feet of plot B was accidentally wet on June 14.

in both the Cavanagh and Peck gardens. Early in September immature fruits in plot C began to loosen from the perianth and drop, and by the middle of September about 23 percent of the fruits (by weight) had dropped in Cavanagh plot C and about 35 percent in Peck plot C. However, this effect of appreciable water deficits in July did not occur in the 1939 Cavanagh experiment or in the 1941 United States Date Garden experiment.

In plot D, where appreciable water deficits in the palm occurred when cell enlargement had ceased and dry weight (mostly sucrose) was increasing rapidly, the rate of dry-matter accumulation was not reduced below that in plot A, at least in the 1939 and 1940 Cavanagh experiments. Thus, the appreciable water deficits in the palm either did not reduce photosynthesis sufficiently to materially diminish the supply of sugars from the leaves to the fruit, or the utilization of stored carbohydrates compensated for any reduction in the supply of sugars from the leaves. That carbohydrates from stored reserves may be utilized late in the development of the fruit has been suggested by Aldrich and Young (*1*), whose results show a decrease in the starch content of the trunk during the period of fruit ripening. In the 1941 United States Date Garden experiment, however, with palms just coming into bearing and with such extremely severe water deficits in one of the three palms in plot D that the fruit shriveled before ripening, the indicated rate of dry-matter increase in the fruit dropped considerably below that in plot A. This suggests that when water shortage is sufficiently acute, the ability of the palm to function normally may be so reduced that the rate of dry-matter accumulation in the fruit is greatly lessened. In all three experiments the normal decrease in the total amount of water per fruit during September was slightly greater in plot D than in plot A.

The extent to which the single periods of appreciable water deficit in the palm limited the final weight of the fruit in plots B, C, and D was calculated (table 2). As would be expected from the weight curves previously shown (fig. 2), appreciable water deficits in the palm in June and July (plots B and C) had a much greater effect in reducing the final fruit weight below that of plot A than did the soil-moisture deficiencies in August and September. Although the effects of midsummer soil-moisture deficiencies in reducing the rates of fresh- and dry-matter increase occurred at different times during the summer, the final fresh and dry weights per fruit were reduced about equally.

TABLE 2.—*Effects of appreciable water deficit in the palm for a single period in limiting the final fresh and dry weights of the fruit*

| Location and year of experiment | Fruit weight obtained | Percentage reduction in weight per fruit in each plot below that of fruit in plot A | | |
|---|---|---|---|---|
| | | Plot B | Plot C | Plot D |
| | | *Percent* | *Percent* | *Percent* |
| Cavanagh garden, 1939 | Fresh [1] | 14 | 12 | 6 |
| | Dry [1] | 16 | 10 | 0 |
| Cavanagh garden, 1940 | Fresh [2] | 6 | 25 | 4 |
| | Dry [2] | 11 | 23 | 2 |
| Peck garden, 1940 | Fresh [3] | ---- | 12 | ---- |
| | Dry [3] | ---- | 16 | ---- |
| United States Date Garden, 1941 | Fresh [4] | ---- | 16 | 9 |
| | Dry [4] | ---- | 13 | 11 |

[1] Ripe fruit in second (October 17) picking.
[2] Ripe fruit in second (September 21) picking.
[3] Partly ripe fruit in September 16 sampling.
[4] Ripe fruit in October 9 sampling.

### FRUIT QUALITY

Checking, the high-humidity or rain injury to which the fruit is susceptible during June, July, and early August, occurred on fewer fruits in plots B and C than in plot A. These results, which are discussed in a separate report (*3*), are believed to be due not only to a reduced supply of water from the trunk to the fruit, but also to physical or chemical changes in the fruit brought about by appreciable water deficits in the palm in plots B and C. Blacknose, a shriveling and darkening of the tips of fruits that develop severe checking, seemed to be reduced by the water deficits proportionately more than the checking.

There is a popular belief among date growers that the severe shrivel of ripe fruits often observed in the Deglet Noor variety is most serious when irrigation is not adequate. Although a careful grading of the fruit in each picking from each plot of all experiments showed that appreciable water deficits in the palm were in some cases followed by a slightly increased amount of shrivel in ripe fruits, there was no convincing evidence that shrivel was greatly increased by a single period of soil-moisture deficiency. Since Nixon and Crawford (*8*) found in fruit-thinning experiments that more shrivel occurred where large numbers of fruits were left on a bunch, it seems probable that shrivel results from a modification of fruit development caused by a limitation in the supply of carbohydrates. Therefore soil-moisture deficiency which is so prolonged as to greatly reduce the vigor of the tree, and perhaps carbohydrates stored in the trunk, might be expected to cause an increase in the number of shriveled fruits.

### SUBSEQUENT DEVELOPMENT OF INFLORESCENCES

The effects of the time of appreciable water deficits in the palm in one year upon the number of inflorescences emerging the following spring (table 3) indicate that the deficits in late August and early September (plot D) resulted in fewer inflorescences (statistically significant in two out of three series) the following year than on the control palms. This suggests that reduced photosynthesis during the appreciable water deficits in the palms limited the carbohydrate or other plant foods necessary for development and emergence of the inflorescences.

TABLE 3.—*Effect of time of appreciable water deficit in the palm upon the number of inflorescences appearing per palm the following spring*

| Location and year of experiment | Inflorescences per palm, in the spring after appreciable water deficits in the palm | | | |
|---|---|---|---|---|
| | Plot A | Plot B | Plot C | Plot D |
| | *Number* | *Number* | *Number* | *Number* |
| Cavanagh garden, 1939 | 13.2±0.7 | 12.8±0.3 | 11.8±0.5 | 9.7±0.6 |
| Cavanagh garden, 1940 | 11.8±0.5 | 11.8±0.7 | 12.0±0.3 | 10.8±0.7 |
| United States Date Garden, 1941 | 20.7±1.9 | ..... | 17.0±0.6 | 10.6±0.9 |

## DISCUSSION

These investigations have shown that systematic measurements of the rate of elongation of date leaves provide a satisfactory method for determining when soil moisture is depleted to such an extent as to

reduce the rate of growth of the fruit. The average rate of leaf elongation of recently emerged leaves was fairly constant from late May to October, so that a decrease of 1 cm. or more per day below this normal rate was an indication of soil-moisture deficiency. Fluctuations in rate from day to day during the summer due to variations in weather would tend to smooth out if the calculated rates were based upon intervals of 6 to 7 days between measurements. This normal summer rate of leaf elongation for Deglet Noor palms with adequate soil moisture was found to be between 4 and 5 cm. per day, with the higher normal rate for the palms of greater vigor. Therefore, with Deglet Noor in the summer, a decrease in rate to about 3.5 cm. or less per day would indicate a soil-moisture deficiency. Under circumstances where the rate of leaf elongation appeared to be lower than normal when measurements were started, an increase in rate of about 1 cm. or more per day immediately after an irrigation would indicate that soil-moisture deficiency had existed before the irrigation. If, on the other hand, a rate of 3.5 cm. or less per day were not increased by an irrigation, some other factor, such as a root disease, should be suspected as limiting the growth of the palm.

To extend this method to other seasons of the year or to varieties other than Deglet Noor, it would be necessary to determine the normal rate of leaf elongation on two or three palms irrigated sufficiently often and thoroughly to have the soil moisture adequate at all times. Such a normal rate would be extremely low in the winter (*2*, *6*), would increase gradually during the spring as air and soil temperatures increase, and after a fairly constant rate during the summer would decrease in the fall. With this normal rate as a basis for comparison, a consistent lower rate of leaf elongation on other palms of the same variety would indicate soil-moisture deficiency.

Of particular importance is the fact that, during the period of increase in fresh weight of fruit (late May, June, July, and early August), a prolonged decrease in rate of leaf elongation of 0.5 to 1 cm. or more per day below the normal rate was always accompanied both by a decrease in the rate of growth of the fruit and by a limitation in its final fresh weight. Furthermore, the extent to which the rate of leaf elongation was reduced below the normal rate as a result of soil-moisture deficiency was approximately proportional to the extent to which the rate of fruit growth was reduced. Therefore, when soil-moisture deficiency is suspected of limiting production, the systematic measurement of the rate of leaf elongation can be used to determine when improper irrigation is retarding the development of the fruit.

Although the results indicate that soil-moisture deficiencies in late August and September would not be expected to reduce the final weight of fruit as much as equivalent deficiencies in June or July, it should not be inferred that such late moisture deficiencies are any less detrimental to satisfactory fruit production in the crop of the following year. The evidence that soil-moisture deficiency in late summer resulted in a smaller number of inflorescences the following spring suggests that such deficiency reduces the vigor of the palm.

Since the period of susceptibility to checking coincides with the period of rapid increase in the fresh weight of the fruit, a soil-moisture deficiency of sufficient magnitude to reduce the susceptibility of the

fruit to severe checking during an extended period of high relative humidity or rain would be expected to markedly reduce its rate of growth. This was true in 1940 at both the Cavanagh and Peck gardens. The plots with soil-moisture deficiencies in June or July had lower percentages of blacknose than the controls, but the fresh weight of the ripe fruits from these plots was 6 to 25 percent less than that of the fruits from the controls. Even if no fruit drop had occurred on palms with a soil-moisture deficiency in July, it is doubtful whether the benefits of reduced blacknose would have increased the market value of the fruit sufficiently to compensate for its reduced size and the consequently reduced yield per palm. In years when blacknose is not serious, as in 1939, the reduction in checking alone as a result of soil-moisture deficiency in June and July would certainly not compensate for the reduction in fruit size.

The development of shrivel of ripe fruits appears to be most serious in years with prolonged periods of high temperature, particularly during September. In such years relatively few fruits develop shrivel where soil and cultural conditions have resulted in vigorously growing palms with numerous large leaves, large fruitstalks, and large fruits. Where the leaves, fruitstalks, and fruits are small as a result of unfavorable soil or cultural conditions, adequate irrigation during one season to prevent soil-moisture deficiency did not keep a fairly large percentage of fruits from developing shrivel that season. Thus the role of careful irrigation in reducing the susceptibility of the fruit to shrivel would seem to be the prevention of soil-moisture deficiencies that might hinder the growth of new leaves or reduce the ability of existing leaves to produce carbohydrates. Since carbohydrate reserves probably accumulate in the fall, winter, and spring (*1*), the prevention of soil-moisture deficiency throughout the year would appear to be desirable.

## SUMMARY

The effects of depleted soil moisture on the rate of leaf elongation and fruit development of bearing date palms of the Deglet Noor variety were determined in four series of experimental plots. The moisture content of the soil was measured at 10-day intervals in one series of plots.

The rate of elongation of recently emerged leaves was found to be fairly constant from late May to October.

A decrease in the rate of leaf elongation below that of adjacent frequently irrigated palms was found to be a sensitive index of appreciable water deficits in the palm resulting from soil-moisture deficiency. A prolonged decrease in rate of leaf elongation of 0.5 to 1 cm. or more per day below that of frequently irrigated palms during late May, June, July, and early August, when the fruits were enlarging, was always accompanied by a reduced rate of increase in fresh weight and by limitation in the final fresh weight of the fruits. The rate of increase in dry weight of the fruits did not fall immediately as much as that of fresh weight, so that the percentage water content of the fruits was temporarily reduced. Delayed effects of appreciable water deficits in the palm were manifested in late summer in a reduced rate of increase in fruit dry weight, in approximately 2 weeks' earlier softening of the fruits, and, during one season, in a premature dropping of the

fruits. Equivalent appreciable water deficits in late August and early September, when the fruits were increasing rapidly in dry matter, resulted in a more rapid decrease in their fresh weight during the normal dehydration just prior to ripening, but there was reduced dry-weight accumulation only where the water deficits in the palm were very severe.

Appreciable water deficits in the palm during June, July, or early August, when the fruits are susceptible to the high relative humidity or rain injuries known as checking and blacknose, resulted in fewer fruits developing these injuries than on palms receiving adequate irrigation. Appreciable water deficits in the palms for a single period in the summer did not greatly increase the shrivel of ripe fruits.

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